A kinetic study of furan formation during storage of shelf-stable fruit juices

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Abstract

To this day, research for furan mitigation has mostly targeted the levels of food production and handling of prepared foods by the consumer. The present study demonstrated the importance of appropriate (moderate) storage conditions for shelf-stable fruit juices, because the furan concentrations of pasteurized orange and mango juices originated from the storage phase, rather than the thermal preservation step. For both juices, the increase in furan concentration during storage was best described by an empirical, logistic model. The duration of the lag phase decreased as a linear function of the storage temperature, while the maximum reaction rate constant followed the Arrhenius law. Furthermore, the rate of furan formation was clearly matrix-dependent, which could be attributed to the differences in juice composition (sugars, ascorbic acid and carotenoids). Next to furan, the juices were also analyzed for 2- and 3-methylfuran. Like for furan, the methylfuran concentrations were matrix-dependent and seemed to increase as a function of storage time and temperature.

Abbreviated running title

Kinetic study of furan formation during storage

Keywords

Furan; methylfuran; fruit juice; pasteurization; storage; kinetics.

Chemical compounds studied in this article

Furan (PubChem CID: 8029)

2-Methylfuran (PubChem CID: 10797)

3-Methylfuran (PubChem CID: 13587)

1. Introduction

Furan (C_4H_4O) is a small organic molecule with high volatility. In 1995, furan was classified as 'possibly carcinogenic' to humans after it was proven to be carcinogenic in rats and mice (International Agency for Research on Cancer (IARC), 1995). Furan can be formed in a variety of heat-treated foods. The highest concentrations are found in coffee products (mean concentrations of up to 45 and 394 µg/kg for brewed and instant coffee, respectively) and canned or jarred foods (32 µg/kg for baby food), products that are subjected to an intensive heat treatment for roasting or sterilization purposes (US Food and Drug Administration (FDA), 2009; European Food Safety Authority (EFSA), 2011). In this context, fruit-based products tend to have a lower furan concentration (5-6 µg/kg for juices and canned fruits) than the above-mentioned products, which is often attributed to the acidity of the systems or the corresponding reduction in the thermal load applied for pasteurization (Bianchi et al., 2006; Zoller et al., 2007; Wegener and Lopez-Sanchez, 2010). Nevertheless, they are important contributors to the furan exposure of almost all consumer groups, and children in particular (European Food Safety Authority (EFSA), 2011). As reflected by the variety of products in which furan has been detected, many reaction pathways leading to the formation of furan are reported in the literature, the major precursors being sugars (alone or in combination with amino acids), ascorbic acid and unsaturated fatty acids, followed by amino acids and carotenoids (Locas and Yaylayan, 2004; Becalski and Seaman, 2005; Fan, 2005; Mark et al., 2006; Limacher et al., 2007; Limacher et al., 2008; Owczarek-Fendor et al., 2010; Owczarek-Fendor et al., 2011; Van Lancker et al., 2011; Huang et al., 2011; Owczarek-Fendor et al., 2012).

Currently, no acceptable intake level is established for furan, since the contaminant is acting as a possible carcinogen through a genotoxic mechanism (Joint FAO/WHO Expert Committee on Food Additives, 2011). Instead, the furan concentrations of heat-treated foods should be kept as low as reasonably achievable (ALARA-principle). When performing a risk analysis of furan, food safety authorities tend to use the lowest dose with an observable, adverse health effect in animal studies. Based on a benchmark dose lower confidence limit ($BMDL_{10}$) of 0.96 mg/kg_{bw} per day, the Joint FAO/WHO

77 Expert Committee on Food Additives (2011) has indicated a human health concern for furan and
78 consequently, actions should be taken to minimize exposure to an acceptable level. To date, research for
79 furan mitigation has mostly been targeted at the levels of food production (Fan and Mastovska, 2006;
80 Sevenich et al., 2014; Palmers et al., 2014) and consumption (Hasnip et al., 2006; Roberts et al., 2008;
81 Kim et al., 2009), both with varying success. The reduction of furan concentrations was found to be
82 challenging, because of the variety of possible furan precursors and the microbial safety standards to be
83 guaranteed. Between the thermal processing step for preservation and final consumption, the product
84 quality is altered by chemical deterioration reactions during storage. Even though many of these
85 reactions occurring during storage are directly related to the formation of furan (e.g. degradation
86 reactions of sugars and ascorbic acid), literature information on the effects of storage for the furan
87 concentrations of different heat-treated foods is scarce. Furthermore, the reported concentrations from
88 monitoring experiments are always a combination of furan concentrations formed during the thermal
89 treatment for preservation and possible changes during storage. For the purpose of furan mitigation, it
90 would be interesting to obtain insight into the contribution of both steps separately. Theoretically, the
91 furan concentration can both show an increase or a decrease during the storage period, as a result of the
92 enhanced degradation of precursors and intermediate products, or furan being degraded itself. In this
93 context, shelf-stable foods might be identified as priority matrices, because of the specific length (up to
94 years) and possible temperatures (room temperature or higher) of storage.
95 The objective of the present study was to investigate the individual effects of the pasteurization
96 treatment and the storage conditions on the furan concentrations of shelf-stable fruit juices. Because of
97 its great commercial relevance, orange juice was selected as an exemplary research matrix. In order to
98 obtain quantitative insight into the effects of the storage parameters time and temperature, a kinetic
99 study was set up. The orange juice was pasteurized and stored at four different temperatures (20-28-35-
100 42 °C), for storage times up to 32 weeks. To verify whether the same storage effect could be observed in
101 other shelf-stable fruit juices, the experiment was partially repeated with mango juice (only at 42 °C).
102 Both juices were analyzed for their initial amounts of important furan precursors such as ascorbic acid,

103 total sugars and total carotenoids, in an attempt to link the changes in furan concentration to the initial
104 composition of the juices. As recently proposed by Becalski et al. (2010) and adopted in the
105 recommendations of the Joint FAO/WHO Expert Committee on Food Additives (2011), the stored
106 orange and mango juices were also analyzed for their concentrations of 2- and 3-methylfuran. Both
107 alkylated derivatives of furan might be of toxicological interest, since animal studies have shown that
108 they can be metabolically activated in a similar way as furan (Gill et al., 2014a; Gill et al., 2014b).
109

110 **2. Materials and methods**

111 *2.1. Preparation of the fruit juices*

112 Orange and mango juices were reconstituted from a frozen concentrate. Single strength orange juice
113 (11 °Brix uncorrected) was obtained from the dilution of Brazilian orange concentrate (65 °Brix
114 uncorrected) with water in a 1:5 ratio (w/w), mango juice (7 °Brix uncorrected) from the dilution of
115 Indian mango purée (17 °Brix uncorrected) in a 1:1 ratio (w/w). Both juices were immediately subjected
116 to an industrially relevant flash pasteurization (92 °C, 30 s) in a tubular heat exchanger. The temperature
117 of the juices was 85 °C when they were filled into polyethylene terephthalate bottles (500 ml). The
118 bottles were cooled to ambient temperature by submerging them in a circulating and chlorinated water
119 tank. Samples were taken for nutrient and (methyl)furan analyses, and the remaining bottles were
120 stored.
121

122 *2.2. Storage conditions*

123 After pasteurization, the fruit juices were placed in incubators for storage at constant temperature, and
124 protected from light. For orange juice, a kinetic study was set up to investigate the changes in furan
125 concentration during storage, by storing the pasteurized juice under a range of time and temperature
126 conditions. To select appropriate storage conditions, the principles of accelerated shelf life testing
127 (Toledo, 2007) were applied. Orange juice was stored at 20 and 28 °C for 32 weeks, at 35 °C for 12
128 weeks, and at 42 °C for 8 weeks. Based on a theoretical temperature coefficient Q_{10} of 2 (Taub and

Singh, 1997; van Boekel, 2009), these selected storage conditions should result in a comparable quality change for the orange juices stored at different temperatures. Mango juice was only stored at 42 °C for 8 weeks, to assess the effect of storage in another shelf-stable fruit juice, and to obtain a first insight into the effect of the juice composition for the changes in furan concentration during storage. The storage temperature for mango juice was selected both from a worst-case perspective and for practical reasons (efficiency of time and storage capability). For both juices, samples for (methyl)furan analysis were removed from the incubators at regular time moments. The samples were transferred to polyethylene terephthalate falcons (50 ml), frozen with liquid nitrogen and stored at -80 °C until analysis.

2.3. Nutrient analyses of the pasteurized juices

The pasteurized fruit juices were analyzed for their concentrations of ascorbic acid, total sugars and total carotenoids, prior to storage. Ascorbic acid analysis was done by high-performance liquid chromatography with photodiode array detection (HPLC-DAD) according to the procedure of Verbeyst et al. (2013). Additional precautions (cooled solutions, protection against light and oxygen) were taken to prevent oxidative degradation of ascorbic acid during the procedures for extraction and analysis. The total sugar content (uncorrected °Brix) was measured at 20 °C using a digital refractometer (RX-7000α, Atago, Tokyo, Japan). The values were not corrected for titratable acids. The total carotenoid concentration was determined based on the procedure of Melendez-Martinez et al. (2007). Carotenoids were extracted from the fruit juices and saponified, to obtain deesterified pigments. After concentration to dryness, the carotenoids were redissolved and analyzed by HPLC-DAD. The total carotenoids content was estimated as the sum of the quantities of the individual carotenoids. Furthermore, the pH of the pasteurized juices was measured with a pH meter (Meterlab PHM210, Radiometer Analytical, Lyon, France), which was calibrated with calibration buffers of pH 4.0 and 7.0 (IUPAC, Radiometer Analytical, Lyon, France). All measurements were done in triplicate.

2.4. Quantitation of furan and methylfuran

155 Furan was analyzed with solid phase microextraction coupled to gas chromatography-mass
156 spectrometry (SPME-GC-MS), using furan-d_4 as an internal standard. The analytical procedure is
157 described in detail elsewhere (Palmers et al., 2014), and will only be discussed here in brief. Standard
158 stock (ca. 2.5 mg/ml in methanol) and working solutions (ca. 0.25 µg/ml in deionized water) of internal
159 standard were obtained by making a serial dilution of furan-d_4 (98%, Sigma Aldrich, Saint Louis,
160 Missouri) in closed headspace vials of 10 ml. Both the amount of solvent and the amount of solution
161 added were determined by differential weighing and the exact concentration of furan-d_4 was calculated
162 from the respective masses. The stored fruit juices were prepared for analysis by weighing 2.5 g of the
163 thawed juice in an empty 10 ml headspace vial with a PTFE/silicone septum seal. Before the vial was
164 completely closed, 2.5 ml of a saturated NaCl solution was added to the juice sample and the mixture
165 was further diluted with deionized water to obtain a standardized total volume of 6 ml. After sealing,
166 100 µl of the internal standard working solution was added with a chilled gastight syringe. The exact
167 amounts were determined again by differential weighing. All operations were done in a closed and
168 refrigerated sample preparation box (MPR-311D(H), Sanyo, Moriguchi, Japan) and the samples were
169 stored at a temperature of 10 °C until analysis. Each time moment was analyzed in duplicate.
170 The analyses were carried out using an Agilent 7890A GC and an Agilent 5975C MS (Keysight
171 Technologies, Santa Rosa, California), equipped with a CTC Combi PAL autosampler (CTC Analytics,
172 Zwingen, Switzerland). The SPME fiber (Supelco, Bellefonte, Pennsylvania) had a 75 µm
173 carboxen/polydimethylsiloxane (CAR/PDMS) sorptive coating, which was exposed to the headspace of
174 the samples for 15 min at 30 °C. After headspace extraction, the fiber was transferred to the GC
175 injection port, where the adsorbed compounds were thermally desorbed for 1 min at 200 °C. After each
176 run, the fiber was thermally cleaned for 2 min at 300 °C in the conditioning station of the autosampler.
177 The volatiles were injected in the splitless mode and subsequently separated on a HP-PLOT Q column
178 (30 m × 320 µm, 20 µm film thickness, Keysight Technologies, Santa Rosa, California), using helium
179 as the carrier gas at a constant flow rate of 2 ml/min. The column oven was programmed at a starting
180 temperature of 40 °C, which was retained for 4 min, after which it was elevated to 160 °C at a rate of 40

°C/min, followed by a second ramp to 220 °C at 5 °C/min. After 1 min at the final temperature, the oven was cooled again to the initial temperature. Mass spectra were obtained by electron ionisation (EI) at 70 eV, in the combined SCAN and SIM mode. The scanning range extended m/z 35-400. The selected ions monitored were m/z 68 (quantifier) and 39 (qualifier) for furan and m/z 72 (quantifier), 44 and 42 (both qualifier) for furan-d_4. MS ion source and quadrupole temperatures were 230 and 150 °C, respectively.

The method of internal standard calibration was used to prepare a calibration curve for furan. A serial dilution was made starting from a furan working solution (ca. 0.25 µg/ml in deionized water), resulting in a calibration curve covering the concentration range of 0-50 ng/g juice. The identity of both furan and furan-d_4 was confirmed by calculating the response ratio of the qualifier ions and the quantifier ions according to the guidelines stated in European Commission Decision (European Commission, 2002). In agreement with these guidelines, the method was validated in terms of specificity, recovery, precision (repeatability), decision limit (CCα), and detection capability (CCβ). The decision limit and the detection capability of the procedure were 1.15 ng and 1.86 ng per g product, respectively. Next to furan, the calibration curve was used to estimate the concentrations of 2- and 3-methylfuran in the samples. According to our own findings (results not shown), the behavior of both methylfurans towards the internal standard furan-d_4 was not significantly different from the behavior of furan. The identity of 2- and 3-methylfuran was confirmed by comparison with retention times of standards and by calculating the response ratio of the qualifier ions and the quantifier ions, as analogous to the identification of furan and furan-d_4. For both methylfurans, the selected ions monitored were m/z 82 (quantifier), 81 and 53 (qualifier).

2.5. Analysis of the kinetic data

Furan formation was kinetically modeled as a function of the storage conditions time and temperature. For a detailed discussion on the general principles of kinetic modeling, the reader is referred to the work of van Boekel (2009). In general, chemical changes in foods are often reported to follow zero- or first-order kinetics, which basically means that the formation or the degradation of the compound under

consideration is (apparently) independent of the reactant concentration or the result of a monomolecular reaction. This observation might hold for a relatively short period of time. However, as the chemical reaction proceeds, the reaction conditions will start to change (e.g. limiting reactant concentrations, pH shift) and the mechanistic basis for the selection of these kinetic models might become blurred. In such a case, the proceeding of the chemical reaction might as well be described by any empirical model that provides an acceptable fit of the data. Given the complexity of the reaction pathways for furan (cf. Introduction) and to describe the complete change in the furan concentration from the start until the end of the storage experiment, an empirical, logistic model was selected in the present study (Equation 1):

$$A = \frac{A_S}{1+exp\left[\frac{4\times k_{max}}{A_S}(\lambda - t)+2\right]} \quad (1)$$

Where A is representing the furan concentration at storage time t, A_s a plateau concentration at long storage times, k_{max} the maximum reaction rate constant, and λ the duration of the lag phase, for a given storage temperature. This logistic model is often used for modeling isothermal growth in microbiology, because the kinetic parameters provide an excellent means for the characterization of sigmoidal curves. The temperature dependence of the kinetic parameters can sometimes be described by theoretically derived or empirical relationships. In the present study, the temperature dependence of the maximum reaction rate constant k_{max} followed the Arrhenius equation (Equation 2), whereas the duration of the lag phase could be described as a linear function of the storage temperature (Equation 3):

$$k_T = k_{ref} exp\left(\frac{-E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right) \quad (2)$$

$$\lambda_T = \lambda_{ref} + b_T\left(T - T_{ref}\right) \quad (3)$$

Where the activation energy E_a and parameter b_T represent a quantitative measure for the temperature-dependencies of the maximum reaction rate constant k_{max} and the lag time λ, respectively. Both temperature relationships were derived empirically, although the Arrhenius equation (Arrhenius, 1889) was afterwards put in a theoretical perspective. For statistical reasons, the storage temperature T was reparameterized against a reference temperature T_{ref}. R is the universal gas constant (8.314 J/K.mol).

The kinetic modeling of the furan concentrations in the stored fruit juices was performed in two steps. First, a suitable kinetic model was selected by visual inspection of the concentration, parity, and residual plots, and by the calculation of $R^2_{adjusted}$. The stored juices were analyzed in duplicate and for each time moment, both data points were entered into the modeling procedure. Second, the corresponding kinetic parameters were estimated using nonlinear one-step regression (SAS version 9.4, Cary, North Carolina).

3. Results and discussion

3.1. Furan formation during pasteurization of orange juice

Orange juice was subjected to an industrially relevant flash pasteurization step (92 °C, 30 s), to obtain a shelf-stable juice for storage. Immediately after pasteurization, the furan level was below the decision limit (<1.15 ng/g juice, cf. Materials and Methods), which in a first approximation corresponds to the detection limit for the analytical procedure of furan. In other words, the amount of furan formed during the thermal pasteurization step for preservation was negligible. Although a matrix effect (e.g. pH of the juices) cannot be excluded, the near absence of furan in the present orange juice can most probably be explained by the relatively mild temperature-time conditions applied for pasteurization. However, in recent monitoring studies by the US Food and Drug Administration (2009) and the European Food Safety Authority (2011), relatively low, but consistent furan concentrations were found in the food category of fruit juices (cf. Introduction). An increase in the furan concentration of pasteurized orange juice during storage would therefore indicate an important role for the storage phase in the furan concentration of commercially available fruit juices.

256 *3.2. Furan formation during storage of orange juice*

257 The furan concentration of the pasteurized orange juice was monitored as a function of storage time at
258 four different temperatures (20, 28, 35 and 42 °C) (**Fig. 1A**). During the first weeks of storage, furan
259 concentrations remained approximately constant at their initial level, i.e. below the decision limit (<1.15
260 ng/g juice). After this lag time, the furan concentration started to increase as a function of storage time.
261 Both the lag time and the rate of furan formation were clearly affected by the storage temperature. At
262 high storage temperatures, the lag phase was shorter and the rate of furan formation was clearly higher.
263 This is an important observation, because it shows that even a small increase in the storage temperature
264 of shelf-stable fruit juices could result in a higher furan concentration. At all temperatures, the increase
265 in furan concentration continued throughout the entire duration of the storage experiment. However,
266 after 24 weeks of storage at 20 or 28 °C, the rate of furan formation slowly decreased, which might
267 suggest that for long storage times, the furan concentration was moving towards a maximum or plateau
268 concentration. To confirm this hypothesis, a specific experimental design with more data points at long
269 storage times would be required, which was outside the scope of this article. The furan concentrations
270 observed after storage (6-9 ng/g juice) were very similar to the concentrations reported in the literature
271 (mean concentration of 5 ng/g juice, 95th percentile of 8-10 ng/g juice) (US Food and Drug
272 Administration (FDA), 2009; European Food Safety Authority (EFSA), 2011). In other words, the
273 present study seemed to demonstrate that the larger part of the furan concentrations found in shelf-stable
274 orange juice originated from the storage phase, rather than the thermal treatment for preservation.

275 For a quantitative discussion of the furan formation during storage of the pasteurized orange juice, the
276 results were analyzed using kinetic modeling. The increase in furan concentrations was best described
277 by an empirical, logistic model with three kinetic parameters (Equation 1). The selected model was
278 reparameterized in order to include the interpretable parameters A_s (hypothetical plateau concentration),
279 k_{max} (maximum reaction rate constant), and λ (duration of the lag phase). At each storage temperature,
280 the kinetic parameters were estimated by means of nonlinear regression and represented in **Table 1**,
281 together with the corresponding value of $R^2_{adjusted}$. **Fig. 1A** shows the fit for the three-parameter logistic

models describing the furan formation at each storage temperature. Next to $R^2_{adjusted}$, model evaluation was based on visual inspection of the parity plot (**Fig. 1B**) and a plot of the residuals (not shown), which all indicated a good fit of the selected models. It should be noted that the logistic models can only be interpreted in a reliable way for the tested range of storage times. In the present storage experiment, there were no data points that prove the actual existence of a plateau concentration for furan. Therefore, it is not possible to attach much significance to the parameter estimates for this plateau concentration. Nevertheless, it is remarkable that the estimates for three out of four storage temperatures (28, 35 and 42 °C) were very close to each other (ca. 9 ng/g juice), which might indicate that there are limiting factors to the furan formation at long storage times (outside the tested range). As opposed to the hypothetical plateau concentration, there was a clear increase in the rate of furan formation for pasteurized orange juice stored at higher temperatures. At each temperature, the rate of furan formation was estimated by its maximum value at the inflection point of the sigmoidal curve. This maximum reaction rate constant nearly doubled for every 7-8 °C temperature increase. At the inflection point of the highest storage temperature tested in this study (42 °C), the furan concentration of the orange juice increased with approximately 1.4 ng/g juice per week. Based on a comparison with the $BMDL_{10}$-value (lowest toxic dose for animals, cf. Introduction), this furan increase does not seem to pose a threat in terms of adverse human health effects. However, a thorough risk evaluation should also consider the general quality of the stored orange juice, and furan exposure through other sources of food. Finally, the logistic model provides a quantitative measure for the lag time at each storage temperature. As already mentioned above, the lag phase can be defined as the first part of the storage experiment, where the furan concentration remained approximately constant at a level below the decision limit (<1.15 ng/g juice). Like for the reaction rate constant, the lag time was clearly dependent on the storage temperature. As the temperature increased, the duration of the lag phase steadily decreased. As a result, there was a difference of over 2 months in the duration of the lag phase at 20 °C (11.0 ± 0.9 weeks) and 42 °C (1.3 ± 0.6 weeks). As mentioned in the Introduction, literature information on the effect of the storage phase for the furan concentrations of heat-treated foods is scarce. In a recent publication by the present authors

(Palmers et al., 2015), the storage effect was investigated for sterilized vegetable purées. Similar to the results of the present study, a clear furan increase was observed for potato and pumpkin purées stored at 35 °C for 5 months. At a refrigerated storage temperature of 4 °C, the furan concentrations of the vegetable purées remained approximately constant for the same period of time, which points to an important effect of the storage temperature. However, a more extensive comparison of both studies is hindered by the distinctly different matrix characteristics and experimental setup. To the best of our knowledge, this is the first study investigating the formation of furan under a wide range of storage conditions. Therefore, it was not possible to check the estimated parameters against other kinetic studies.

The maximum reaction rate constant and the duration of the lag phase were clearly dependent on the storage temperature. In order to investigate the temperature relationship of both kinetic parameters, the parameter estimates obtained in the logistic models (**Table 1**) were plotted as a function of the storage temperature. The temperature dependence of the maximum reaction rate constant followed the Arrhenius law (**Fig. 2A**), whereas the lag time could be described as an inverse, linear function of the storage temperature (**Fig. 2B**). By combining the expressions for the temperature relationship of both kinetic parameters (Equations 2 and 3, respectively) with the three-parameter logistic model described above (Equation 1), an overall kinetic model was obtained that described the furan formation in pasteurized orange juice as a function of the storage temperature and time. Global fitting improved the precision of the parameter estimates, because the parameters are shared over all the individual datasets (van Boekel, 2009). The overall logistic model consisted of five kinetic parameters, expressed against a reference temperature of 28 °C. The kinetic parameters were estimated by means of nonlinear one-step regression and represented in **Table 2**, together with their explanation for the furan formation in pasteurized orange juice during storage. Visual inspection of the parity and residual plots (graphs not shown) and the calculation of $R^2_{adjusted}$ (0.9836) all indicated a good fit of the overall logistic model. The hypothetical plateau concentration was estimated at 8.87 ± 0.61 ng/g juice, which was in line with the individual estimates at 28, 35 and 42 °C. Like for the three-parameter logistic models described above,

334 the estimate for the plateau concentration should be interpreted with care. Even though it was not
335 actually observed in the present storage experiment, this kinetic parameter is an inevitable part of the
336 logistic model. Since there was no clear temperature relationship observed for the plateau concentrations
337 estimated with the three-parameter logistic models, this kinetic parameter was included in the overall
338 model as a fixed level. The maximum reaction rate constant and the lag time for the overall model were
339 estimated at the reference temperature of 28 °C. The corresponding estimates at each individual storage
340 temperature can be calculated from these reference values, but the results will not be discussed here
341 again, because of the strong similarities with the parameter estimates obtained for the three-parameter
342 logistic models. However, please note that the 95 % confidence intervals for the parameter estimates
343 obtained in the overall model are markedly smaller as compared with the estimates from the individual
344 three-parameter models. Finally, the overall logistic model also provided a quantitative measure for the
345 temperature-dependencies of the maximum reaction rate constant (activation energy of 69.8 ± 7.4
346 kJ/mol) and the lag time (decrease of 0.46 ± 0.07 weeks/K^{-1}), which were at least in the same range as
347 other chemical reactions in foods (van Boekel, 2009).

348 The logistic models of the present study can be a tool for design and optimization of the storage step,
349 but the kinetic parameters should always be established specifically for the product under consideration.
350 Furan formation is the result of many interacting, complex reaction pathways rather than a single
351 conversion step. As a result, the observed reaction rate constant is a composite one, strongly influenced
352 by intrinsic matrix properties (e.g. precursors, pH, redox condition). The empirical nature of the logistic
353 models did not allow to obtain any mechanistic insight into these complex reaction pathways leading to
354 furan formation in the pasteurized orange juice during storage. Possible furan precursors in orange juice
355 include ascorbic acid, sugars, and carotenoids (**Table 3**). In the literature, the degradation of these furan
356 precursors has frequently been reported in the context of non-enzymatic browning of pasteurized orange
357 juice (Yeom et al., 2000; Min et al., 2003; Bull et al., 2004; Cortes et al., 2008). This brown
358 discoloration of orange juice was also observed in the present study, especially at long storage times and
359 high storage temperatures. In this context, it should be noted that polyethylene terephthalate (PET)

360 bottles were used for storage of the juices. This type of packaging material does not completely exclude
361 oxygen from entering the headspace of the product. Therefore, throughout the entire duration of the
362 storage experiment, a small amount of oxygen was available for oxidative degradation of ascorbic acid
363 and carotenoids. For unambiguous identification of the main furan precursors during storage, a specific
364 experimental design should be set up (e.g. using labeled precursors or addition of known precursor
365 concentrations). However, the results of this section demonstrated that the storage temperature and time
366 provide a strong means for the control of furan formation in pasteurized orange juice during storage.
367
368 *3.3. Furan formation during pasteurization and storage of mango juice*
369 The storage experiment which was performed for orange juice (Section 3.2), was partly repeated with
370 mango juice. This way, the observed effect of storage was verified in a shelf-stable juice with different
371 composition. The mango juice was subjected to an identical flash pasteurization step as for orange juice
372 (92 °C, 30 s) and stored at 42 °C for 8 weeks. The furan concentration of the pasteurized mango juice
373 was monitored as a function of storage time (**Fig. 3**). Immediately after pasteurization, the level of furan
374 concentration was below the decision limit (<1.15 ng/g juice). The furan concentration then slowly
375 increased until the end of the storage experiment, reaching a maximum concentration of 5 ng/g juice.
376 Since the furan formation in the stored mango juice followed a similar course as for the orange juice, the
377 results confirmed the importance of the storage phase for the furan concentration of shelf-stable fruit
378 juices. It should be noted that the pasteurized mango juice was only stored at one (temperature-abuse)
379 condition of 42 °C. Such high storage temperatures might occur in the case of an (often unintentional)
380 temperature increase, for example during the storage of shelf-stable fruit juices in retail or (sub)tropical
381 regions. For a detailed characterization of the furan formation in mango juice stored at different
382 temperatures, a more elaborate kinetic study should be set up, analogously to the storage experiment
383 performed for orange juice. However, because of the very similar nature of both matrices, it can be
384 reasonably assumed that the furan formation at moderate temperatures (e.g. room temperature) would
385 occur at a lower rate than the furan formation at 42 °C. In order to maintain the furan concentrations at

386 their low initial levels, the storage conditions of the pasteurized juices should therefore be closely
387 monitored and an (accidental) increase of temperature should be avoided as much as possible.

388 Like for the orange juice, the observed increase in furan concentration during storage of mango juice
389 was best described by an empirical, three-parameter logistic model (Equation 1). The estimated kinetic
390 parameters are represented in **Table 1**, together with the estimates for orange juice. Parity, residual plots
391 (graphs not shown) and the calculation of $R^2_{adjusted}$ (0.9977) all indicated a good fit of the selected
392 model. An overlay of the logistic models describing furan formation in the orange and mango juices
393 stored at 42 °C is shown in **Fig. 3**. Even though the lag time for both types of juices was almost identical
394 (ca. 1 week), the rate of furan formation in mango juice (0.760 ± 0.085 ng/g juice/week) was about two
395 times smaller as compared with orange juice (1.414 ± 0.401 ng/g juice/week). This observed difference
396 in the rate of furan formation was supported by the results of a post hoc Tukey test (significance level
397 set at $\alpha = 0.05$) for pairwise comparison of the furan concentrations, which confirmed that the orange
398 juice had a significantly higher furan concentration than the mango juice at the three longest (common)
399 storage times. Since the applied pasteurization process, packaging material and storage conditions were
400 identical for both types of fruit juices, the reason for this difference must be looked for in the intrinsic
401 matrix properties. Oxidative deterioration reactions such as the degradation of ascorbic acid and
402 carotenoids might have an important contribution to the furan formation during storage. Results of
403 nutrient analyses showed that the mango juice contained lower levels of ascorbic acid, sugars and
404 carotenoids as compared with the orange juice (**Table 3**). Similar to the orange juice, the pasteurized
405 mango juice was also characterized by a brown discoloration during storage. However, the brown color
406 was less pronounced, even after 8 weeks of storage at 42 °C. Based on these observations, it can be
407 hypothesized that the furan increase was less pronounced in the mango juice, because there was a
408 smaller amount of furan precursors to be degraded. Again, it should be noted that the formation of furan
409 is the result of a complex network of reactions and interactions. Other matrix characteristics such as pH
410 and redox condition will also have an influence on the degradation reactions to furan. The effect of the
411 matrix composition on the extent of furan formation during storage should therefore be subject to future

research. Intervention in the reaction pathways leading to furan formation, might offer complementary solutions for furan mitigation in shelf-stable fruit juices. For example, glass bottles might have the advantage of preventing oxygen from entering the headspace of the product, thus resulting in a reduced rate of furan formation during storage.

3.4. Trends on the methylfuran concentrations of orange and mango juices

Next to furan, the stored orange and mango juices were also analyzed for 2- and 3-methylfuran. Due to the little amount of literature data on the toxicological relevance and the reaction pathways leading to the formation of both methylfurans, the discussion will be limited to a qualitative description (no kinetic modeling) of the changes in methylfuran concentrations during storage and their significance for furan mitigation. The concentrations of both alkylated furan derivatives were very similar to each other. To facilitate their discussion, the concentrations of both compounds were summed together as a group of methylfurans. Negligible amounts of methylfuran were formed during the thermal processing step for pasteurization. For both juices, the initial levels of 2- and 3-methylfuran were below the decision limit (<1.15 ng/g juice). In the pasteurized orange juice (**Fig. 4**), the summed methylfuran concentrations slowly increased as a function of the storage temperature and time. After approximately 24 weeks of storage at 20 or 28 °C, the increase leveled off at a plateau concentration of 2-3 ng/g juice. Slightly higher concentrations (5-6 ng/g juice) were obtained at 35 and 42 °C, where the increase in methylfuran concentrations continued until the end of the storage experiment. In this case, the summed methylfuran concentrations were almost equal to the corresponding furan concentrations (6-9 ng/g juice). In general, methylfuran formation during storage of the pasteurized orange juice seemed to follow similar trends as for furan. Moreover, important juice constituents and possible furan precursors such as ascorbic acid, sugars, and carotenoids are also reported to form methylfuran in the literature (Becalski and Seaman, 2005; Mark et al., 2006; Limacher et al., 2007; Limacher et al., 2008). The pasteurized mango juice was characterized by a slow increase in the methylfuran concentrations compared with orange juice. After 8 weeks of storage at 42 °C, the summed methylfuran concentration in mango juice amounted to 2 ng/g

juice. The lower methylfuran concentrations in mango juice could possibly be explained by the lower amounts of ascorbic acid, sugars and carotenoids as compared with orange juice. As can be seen from these results, the extent of methylfuran formation during storage of pasteurized fruit juices was clearly dependent on both the storage conditions and the type of matrix under consideration. For the same reasons as furan, it is advised to closely monitor the storage conditions of pasteurized fruit juices. The current knowledge on the mechanism of methylfuran formation is inadequate to fully understand and predict the methylfuran concentrations in fruit juices based on the present results. If necessary, furan and methylfuran concentrations can most likely be reduced by limiting the storage temperature and/or time. Reducing methylfuran concentrations by changing the composition of the product is more challenging with the current lack of information on the formation of both compounds.

4. Conclusions

The present study demonstrated the importance of appropriate storage conditions for furan mitigation in pasteurized fruit juices, because the furan concentrations of orange and mango juices originated from the storage phase, rather than the thermal processing step for preservation. Immediately after pasteurization, the furan levels of both juices were below the decision limit. After a certain lag time, the furan concentration started to increase as a function of the storage temperature and time. The increase in furan concentration was best described by an empirical, logistic model. The selected model was reparameterized to include quantitative measures for the duration of the lag phase, the maximum reaction rate constant, and a hypothetical plateau concentration for long storage times. The lag time decreased as a linear function of the storage temperature, while the maximum reaction rate constant followed the Arrhenius law. Next to the observed effect for the storage conditions, the rate of furan formation was clearly matrix-dependent. At a fixed storage temperature, the rate of furan formation was twice as fast in orange juice as compared with mango juice. The matrix-dependency could most probably be explained by differences in the juice composition regarding furan precursors (ascorbic acid, sugars, and carotenoids). In this context, it would be interesting to check whether other types of food

products have the same tendency to form furan during storage. In view of their possible toxicological relevance to the risk characterization of furan, the stored orange and mango juices were also analyzed for 2- and 3-methylfuran. The observed methylfuran concentrations were in the same order of magnitude and followed comparable trends as for furan. However, with the current lack of information on the reaction pathways leading to methylfuran formation, it remains difficult to control their concentrations during storage. In anticipation of more information on the toxicological effects and the formation of both methylfurans, it would therefore be relevant to take all concentrations (furan and 2- and 3-methylfuran) into consideration when performing a total risk analysis of furan. Meanwhile, it is advised to closely monitor and control the storage conditions of ready-to-eat foods, in order to avoid the formation of unnecessary amounts of furan and methylfuran during storage. If deemed necessary, a kinetic study (as performed in this study) can be set up to identify appropriate, moderate storage conditions for a specific product.

Acknowledgements

Stijn Palmers and Miete Celus are funded by the Agency for Innovation by Science and Technology in Flanders (IWT), Tara Grauwet by the Research Foundation Flanders (FWO), and Biniam T. Kebede by the KU Leuven Research Fund.

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List of tables

Table 1. Parameter estimates with 95 % confidence interval for the empirical, three-parameter logistic model (Equation 1) describing furan formation in the pasteurized orange and mango juices at different storage temperatures.

Matrix	Storage temp. (°C)	A_s (ng/g juice)	k_{max} (ng/g juice/week)	λ (weeks)	Adj. R^2
Orange juice	20	4.21 ± 0.35	0.194 ± 0.015	11.0 ± 0.9	0.9966
Orange juice	28	9.75 ± 0.91	0.449 ± 0.053	7.9 ± 1.4	0.9931
Orange juice	35	8.98 ± 2.78	0.795 ± 0.108	4.1 ± 1.0	0.9923
Orange juice	42	8.28 ± 1.28	1.414 ± 0.401	1.3 ± 0.6	0.9860
Mango juice	42	5.78 ± 0.60	0.760 ± 0.085	0.5 ± 0.3	0.9977

Table 2. Parameter estimates with 95 % confidence intervals for the five-parameter logistic model (i.e. combination of Equations 1, 2 and 3) describing furan formation in the pasteurized orange juice during storage. The kinetic parameters were estimated by means of nonlinear one-step regression, using a reference temperature of 28 °C.

Kinetic parameter	Parameter estimate	Explanation
A_s	8.87 ± 0.61 ng/g juice	Hypothetical plateau concentration
k_{ref}	0.445 ± 0.039 ng/g juice/week	Maximum reaction rate constant at T_{ref} = 28 °C
E_a	69.8 ± 7.4 kJ/mol	Activation energy
λ_{ref}	8.0 ± 1.0 weeks	Duration of the lag phase at T_{ref} = 28 °C
b_T	-0.46 ± 0.07 weeks/K^{-1}	Temperature-dependency of the lag phase

603 **Table 3.** Results of the nutrient analyses (ascorbic acid, total sugars and total carotenoids) and pH
604 assessment for the pasteurized orange and mango juices prior to storage. All the results are represented
605 as means ± standard deviations.

Juice characteristic	**Orange juice**	**Mango juice**
Ascorbic acid (mg/l)	476.18 ± 15.68	16.98 ± 0.24
Total sugars (uncorrected °Brix)	10.86 ± 0.01	7.28 ± 0.01
Total carotenoids (mg/l)	6.49 ± 0.57	0.03 ± 0.01
pH	3.72 ± 0.01	4.06 ± 0.01

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608 **List of figures**
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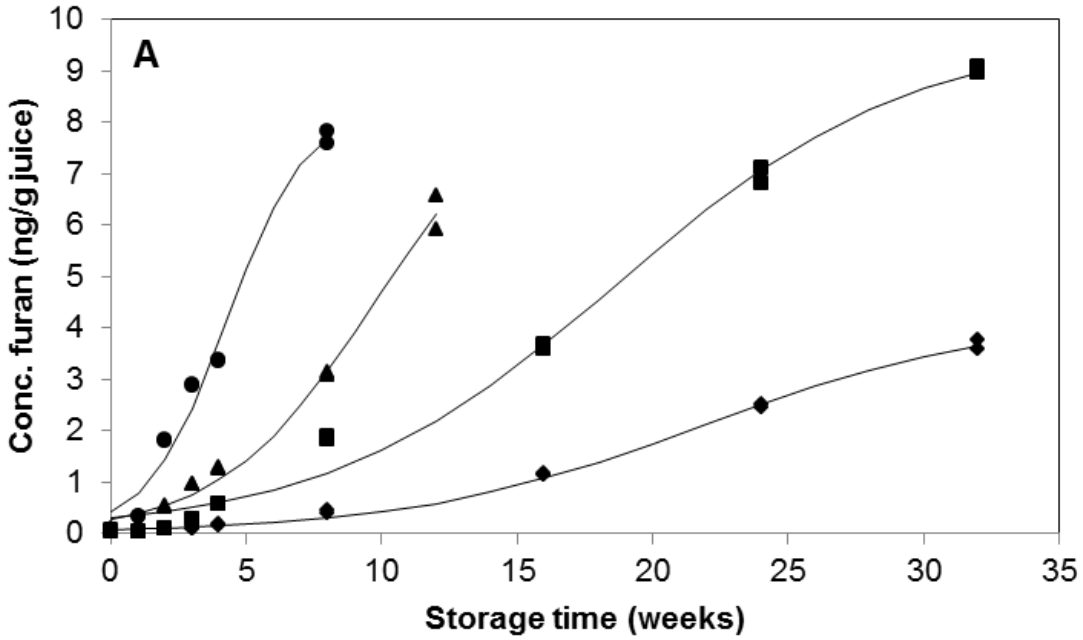

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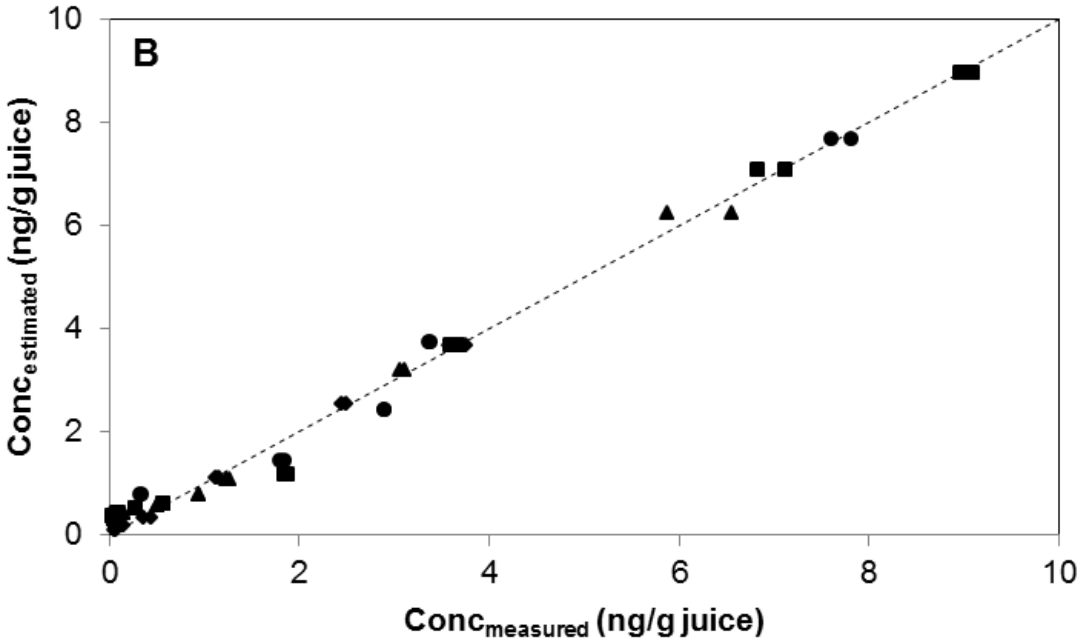

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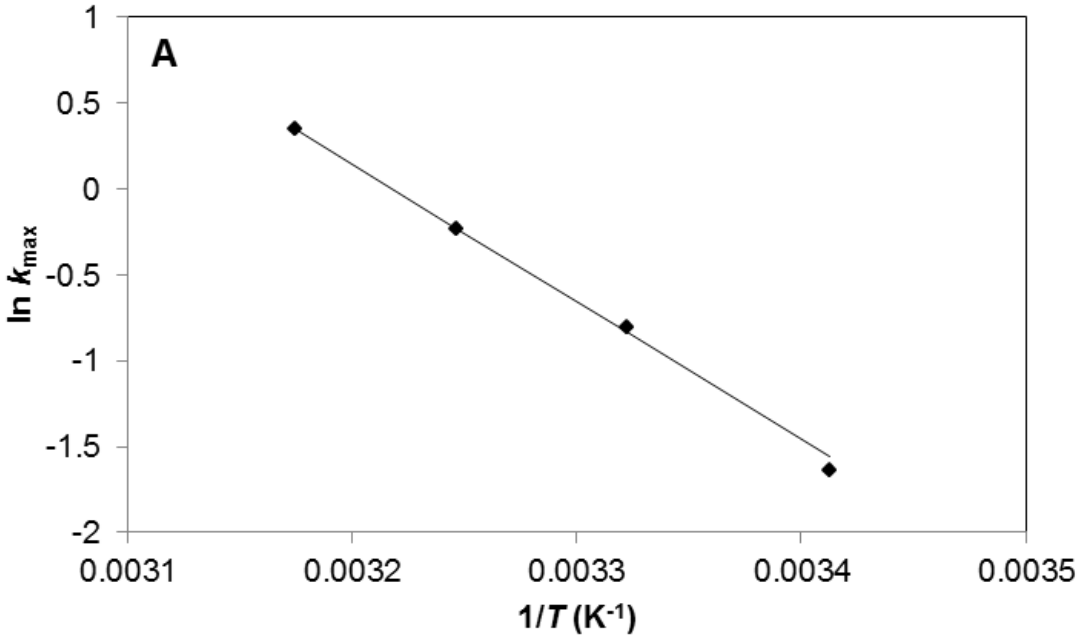
612 **Fig. 1.** (A) Furan concentration in the pasteurized orange juice as a function of storage time at 20 (♦), 28
613 (■), 35 (▲) and 42 °C (●). The full lines represent the furan concentrations estimated by an empirical,
614 three-parameter logistic model (Equation 1), while the measured concentrations are represented by the
615 symbols. (B) Parity plot of the estimated versus the measured furan concentrations.



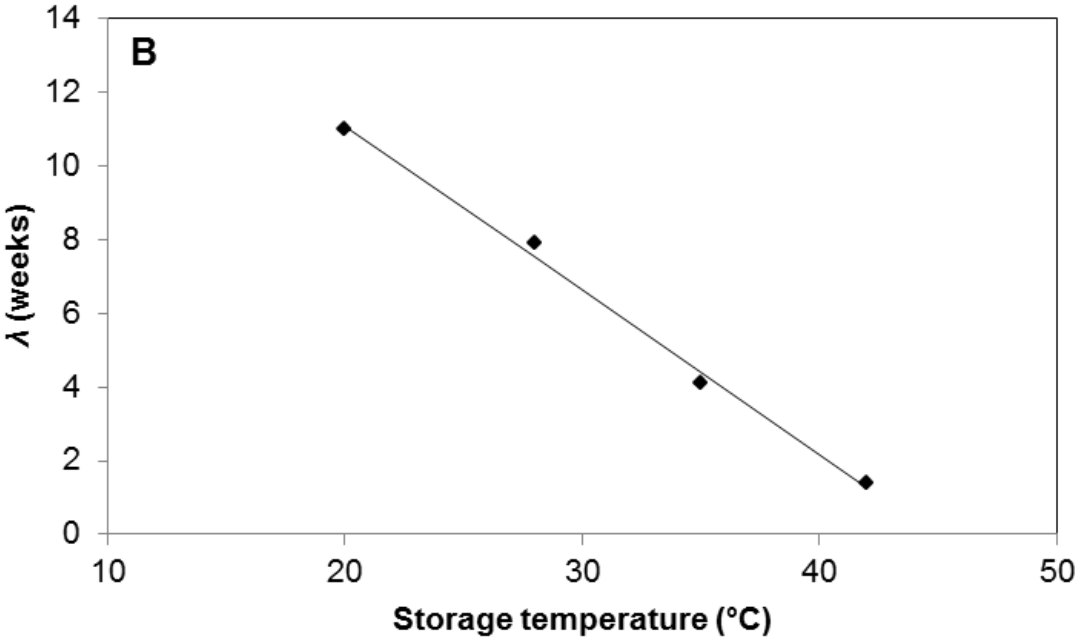
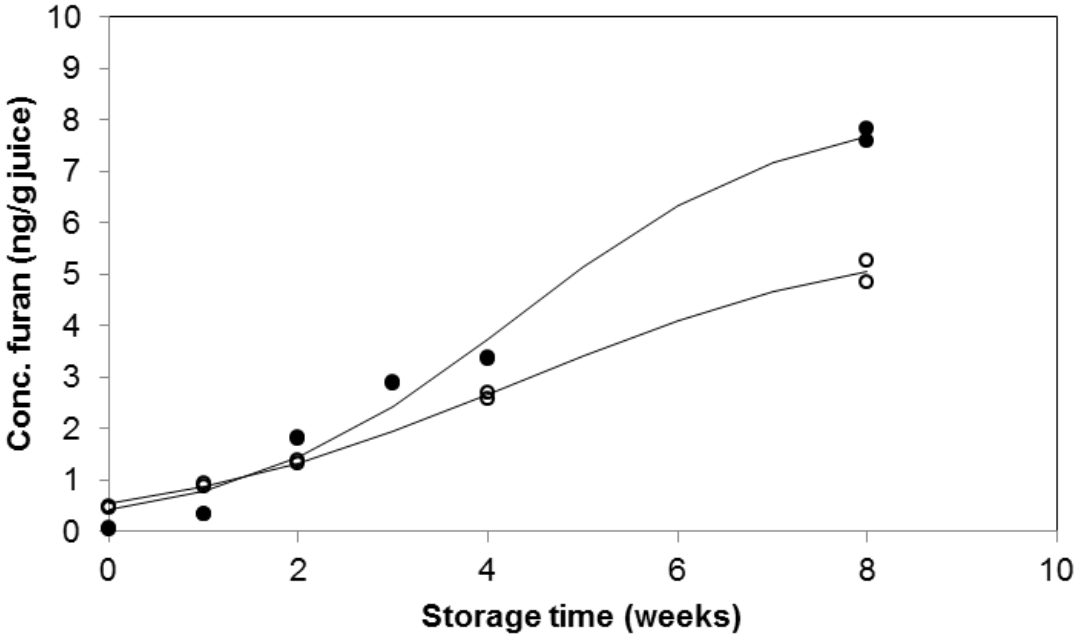


Fig. 2. (A) Arrhenius plot of the maximum reaction rate constants (k_{max}) for furan formation during storage of the pasteurized orange juice, estimated by a three-parameter logistic model. (B) Duration of the lag phase (λ) as estimated by the same logistic models, as a function of the storage temperature. The full line describes an inverse, linear relationship for the temperature dependence of the lag time.



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624 **Fig. 3.** Overlay of the furan concentration in the pasteurized orange (●) and mango (○) juices as a
625 function of storage time at 42 °C. The full lines represent the furan concentrations estimated by an
626 empirical, three-parameter logistic model (Equation 1), while the measured concentrations are
627 represented by the symbols.

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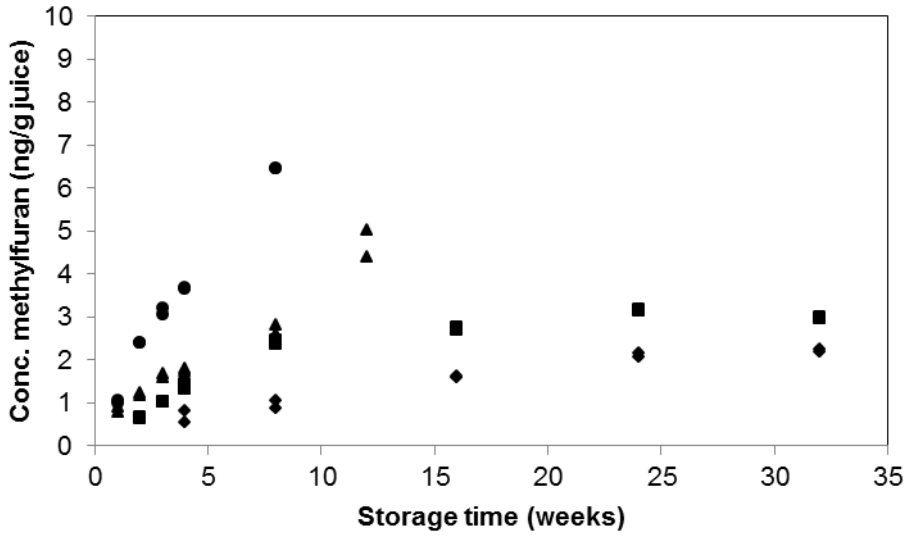

629

630 **Fig. 4.** Concentration of summed methylfuran in the pasteurized orange juice as a function of storage
631 time at a temperature of 20 (♦), 28 (■), 35 (▲) and 42 °C (●).